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Screening of selected species from Spanish flora as a source of bioactive substances

Milan S. Stanković [a,*], Zorica Stojanović Radić [b], José Blanco-Salas [c], Francisco M. Vázquez-Pardo [c], Trinidad Ruiz-Téllez [d]

[a] Department of Biology and Ecology, Faculty of Science, University of Kragujevac, Radoja Domanovića str., 12, 34 000 Kragujevac, Serbia
[b] Department of Biology and Ecology, Faculty of Science and Mathematics, University of Niš, Višegradska str., 33, 18 000 Niš, Serbia
[c] Grupo HABITAT, Instituto de Investigaciones Agrarias Finca La Orden-Valdesequera, CICYTEX, Junta de Extremadura, Autovía A-5 Km 372, 06187 Guadajira, Badajoz, Spain
[d] Grupo de Investigación en Biología de la Conservación, Facultad de Ciencias, Universidad de Extremadura, Avda, de Elvas s/n, 06071 Badajoz, Spain

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ABSTRACT

In the presented study, a comparative analysis of phenolic compounds, parallel with antioxidant and antimicrobial activity of selected plants from Spanish medicinal flora was conducted. Total phenolic content, flavonoid concentrantions, antioxidant activity and antioxidant capacity, as well as antimicrobial activity were determined for methanolic extracts from 21 medicinal or potential medicinal plants sampled from eight different locations on the teritory of Spain. The phenolic content, antioxidant properties and antimicrobial activity were determined for the first time for some analyzed species. Results for total phenolic content determined using Folin-Ciocalteu reagent and expressed in term of gallic acid equivalent, GAE (mg of GA/g of extract) ranged from 35.67 to 286.18 mg of GA/g of extract. The concentrations of flavonoids determined using spectrophotometric method with aluminium chloride and expressed in terms of rutin equivalent, RuE (mg of Ru/g of extract) ranged from 31.58 to 147.34 mg of Ru/g of extract. Antioxidant activity was determined using DPPH (1,1-diphenyl-2-picrylhydrazyl radical) reagent and was expressed as IC_{50} (concentration of sample which is required to scavenge 50% of radicals), where values ranged from 123.80 to 14.77 μg/ml. Obtained results for antioxidant capacity evaluated using the Briggs-Rauscher oscillating reaction method ranged from 190.00 to 19.00 min. Antimicrobial activities of the studied methanol extracts were determined against three gram positive, three gram negative and two fungal strains using microdilution method. Investigated extracts exhibited variable activities with minimal inhibitory concentrations ranging from 0.01-20.00 mg/ml. A significant relation was noticed between the phenolic content, antioxidant activity analyzed through both methods and antimicrobial activity. High amounts of phenolics responsible for the good biological activity of the extracts from the tested plants is in connection with the local, zonal imposed habitat characteristics. The findings of our comparative study and interpretations of the obtained results promote the Mediterranean medicinal flora as an effective source of bioactive substances.

1. Introduction

The Spanish vascular plant flora is composed of 204 families and 7071 species (Aedo et al., 2013), among which approximately 10% belong to the group of medicinal plants (Font, 2014). Especially important are the families Lamiaceae, Apiaceae, Fabaceae, Solanaceae, Scrophulariaceae, Ranunculaceae and Asteraceae. Those having essential oils have been traditionally used not only as terapeutic agents, but also as culinary ingredients, mostly in the mediterranean part of the country.

The biggest part of the Spanish territory has a mediterreanean bioclimate (Martínez, 2015), characterized by a dry summer that is not present at regions with temperate bioclimate. Beside the fact that climate determines the specificity of quantitative and qualitative composition of flora, dry conditions resulted with many plant adaptations at morphological, anatomical, physiological, cellular and biochemical level, along the evolutionary process. Thus, mediterranean species, as well as other plants from arid environments (Stanković et al., 2015), have chemical components that are

* Corresponding author.
E-mail address: mstankovic@kg.ac.rs (M.S. Stanković).

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interpreted as a response to the high insolation and hydric stress, typical for the mediterranean bioclimates.

Plant secondary metabolites, as products of specifically conceived secondary metabolism, which is a continuation of the essential primary metabolism, has a main role in their interaction in the environment. Quantitative and qualitative composition of secondary metabolites, in particular plant organs, tissues, as well as the life cycle of the plant is in accordance with a variety of environmental abiotic and biotic factors (e.g. Oh et al., 2009).

In addition to the role in the process of interaction and adaptation of plants, secondary metabolites isolated from plants exhibit a number of *in vitro* and *in vivo* biological effects. The mechanism of their activity in biological systems is the stimulation or inhibition of enzymatic activity, as well as other metabolic processes, which is the basis of their therapeutic effectiveness in human organism (e.g. Faggio et al., 2015a,b; Faggio et al., 2016; Korkina, 2007; Trischitta and Faggio, 2006, 2008).

Essential oils, a chemical mixtures where phenolic compounds commonly occur, are a good example of this. These molecules, as well as flavonoids, very often exhibit antioxidant activity, which has been related to the level of the molecules resonance (Hortigón-Vinagre et al., 2014). On that basis, the results of the study of secondary metabolites of plants and their biological characteristics in each case have the possibility of a scientific and practical application: clarification of plant adaptive abilities, as well as proposed therapeutic applications in the pharmaceutical and other industries.

Considering the importance of research on biological activity in relation to the amount of secondary metabolites, as well as the effectiveness of comparative analysis of a larger number of samples, 21 plants from eight families from Spanish wild flora were selected for this study. The chosen plant group is characterized by differences in horology, the status of endemism, application in folk and modern medicine and cuisine, as well as in the existence of literature data of previous research. Therefore, the purpose of this comparative screening was to explore part of Spanish flora as a source of phenolic compounds with antioxidant and antimicrobial potential in order to contribute to the knowledge of previous traditional use, as well as to discover novel effective sources of biological active substances. To achieve these objectives, total phenolic content parallel with flavonoid concentration, as well as antioxidant and antimicrobial potential of selected plant species were determined.

2. Material and methods

2.1. Plant material

Total of 21 plants with different representation in herbal medicine as well as area of distribution were collected at their full flowering stage during 2013. The studied species belong to six plant families. Plants were collected from eight different locations on the teritory of Spain – Iberian Peninsula (Table 1). The voucher specimens were confirmed by Dr. J. Blanco Salas and Prof. T. Ruiz Téllez, University of Extremadura and deposited at the Herbarium (HSS) of the "Centro de Investigacion La Orden-Valdesequera, Junta de Extremadura" in Guadajira (Badajoz, Spain). Plant material was air-dried in the dark, at ambient temperature. Air-dried material was milled in a grinder and stored in tightly sealed dark containers until the analysis.

2.2. Chemicals

Organic solvents and sodium hydrogen carbonate were purchased from "Zorka pharma" Šabac, Serbia. Gallic acid, rutin hydrate and 2,2-dyphenyl-1-picrylhydrazyl (DPPH) were obtained from Sigma Chemicals Co., St Louis, MO, USA. Folin-Ciocalteu phenol reagent and aluminium chloride hexahydrate ($AlCl_3 \times 6H_2O$) were purchased from Fluka Chemie AG, Buchs, Switzerland. All other solvents and chemicals were of analytical grade.

2.3. Preparation of plant extracts

Prepared plant material (10 g) was transferred into dark-coloured flasks, filled with 200 ml of methanol and stored at room temperature. After 24 h, infusions were filtered using Whatman No. 1 filter paper and residue was re-extracted with an equal volume of solvents. After 48 h, the process was repeated. Combined supernatants were evaporated to dryness under vacuum at 40 °C using rotary evaporator. The obtained extracts were kept in sterile sample tubes and stored in a refrigerator at 4 °C.

2.4. Determination of total phenolic contents

The total phenolic content was determined using the spectrophotometric method (Mihailović et al., 2015). The reaction mixture was prepared by mixing 0.5 ml of methanolic solution (1 mg/ml) of extract, 2.5 ml of 10% Folin-Ciocalteu's reagent dissolved in water and 2.5 ml 7.5% $NaHCO_3$. The samples were incubated at 45 °C for 15 min. The absorbance was measured at λ_{max} = 765 nm. The samples were prepared in triplicate and the mean value of absorbance was obtained. Blank was concomitantly prepared, with methanol instead of extract solution. The same procedure was repeated for the gallic acid and the calibration line was construed. The total phenolic content was expressed in terms of gallic acid equivalent (mg of GA/g of extract).

2.5. Determination of flavonoid concentrations

Concentration of flavonoids was determined using the spectrophotometric method (Mihailović et al., 2015). The sample contained 1 ml of methanolic solution of the extract in the concentration of 1 mg/ml and 1 ml of 2% $AlCl_3$ solution dissolved in methanol. The samples were incubated for an hour at room temperature. The absorbance was measured at λ_{max} = 415 nm. The samples were prepared in triplicate and the mean value of absorbance was obtained. The same procedure was repeated for the rutin and the calibration line was construed. Concentration of flavonoids in extracts was expressed in terms of rutin equivalent (mg of Ru/g of extract).

2.6. Evaluation of DPPH scavenging activity

The ability of the plant extract and reference substance to scavenge DPPH (1,1-diphenyl-2-picrylhydrazyl radical) free radicals was assessed using the spectrophotometric method (Stanković et al., 2015). The stock solution of the plant extract was prepared in methanol to achieve the concentration of 1 mg/ml. Dilutions were made to obtain concentrations of 500, 250, 125, 62.5, 31.25, 15.62, 7.81, 3.90, 1.99, 0.97 μg/ml. Diluted solutions (1 ml each) were mixed with 1 ml of DPPH methanolic solution (80 μg/ml). After 30 min in darkness at room temperature (23 °C), the absorbance was recorded at 517 nm. The control samples contained all the reagents except the extract. The percentage inhibition was calculated using the equation: % inhibition = 100 x (A *of control* – A *of sample*)/A *of control*), whilst IC_{50} values were estimated from the% inhibition versus the concentration sigmoidal curve, using a non-linear regression analysis. In presented results, antioxidant efficiency of the extract increased with the decreasing of IC_{50} val-